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Original Article

THE EFFECT OF CIGARETTE SMOKING ON CORNEAL ENDOTHELIAL CELLS

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Abstract

Purpose: *To reveal the effect of cigarette smoking on corneal endothelial cells.* ***Patients and methods***: *this is a case control cross sectional study including 99 persons, 61 chronic cigarette smokers and another age matched 38 control nonsmokers. Parameters used in our study were cell density (CD), polymegethism, pleomorphism, the average of cell size, and central corneal thickness (CCT).* ***Results***: *The mean values of the CD were 2829.9±334.9 compared to 3243.6±237.9, CV were 44.13±9.73 compared to 32.84±2.97, HEX were 40.44±6.95% compared to 60.11±5.83% for smokers and non-smoker groups respectively, with a highly statistically significant value (p <0.001) and on the other hand values were statistically non-significant regarding CCT. There were non-significant relation between the type of smoking (cigarette, shisha or combined).* ***Conclusion***: *Cigarette smoking has deteriorating effects on some, but not all, of the corneal endothelial measures, with no relation found between this deteriorating effect and both of the dose or type of smoking.*

Keywords: *Smoking, Corneal endothelial cells, Cell density, Coefficient of variation, Hexagonality*

1. Introduction

The cornea is the transparent front part of the eye and contributes estimated two-thirds of the optical power. Human cornea is mainly composed of corneal epithelium (the outer layer), stroma (the middle layer), and endothelium (the inner layer). Normal morphologies and functions of these cells maintain the transparency of the cornea [1,2]. The corneal endothelium is a monolayer lining the posterior surface of the cornea and is responsible for keeping the cornea in a dehydrated state [3]. The ocular surface mucosa (mainly the cornea) is the first layer of the eye that is exposed to environmental stress. The cornea is susceptible to be damaged by varieties of external stresses due to its constantly direct exposure to harmful factors, such as physical or chemical injuries, UV radiation, and air pollutants (gases, vapors, or cigarette smoke) [1,2].Cigarettes Contain more than 4000 toxic substances which are associated with many cardiovascular, respiratory and malignant disorders [3, 4]. Also, cigarette smoking can cause many ophthalmological disorders as primary open angle glaucoma(POAG), diabetic retinopathy (DR), dry eye disease (DED), optic neuritis (ON), thyroid ophthalmo-

pathy, age related macular degeneration (ARMD) and ocular inflammation [3-6]. Smoking also affects the eyes by impairing blood flow or facilitating the formation of clots within ocular capillaries, thus cutting off vital nutrients that are essential for eye health [7]. Furthermore, passive smoking has been associated with ocular diseases such as hypermetropia, dry eye disease, and cataract [8, 9]. The effect of smoking on the cornea has been studied, Altinors et al have reported that smoking damages precorneal tear film lipid layer and causes dry eye symptoms [10]. Zoega et al found that smoking more than 20 packs per year increase the risk of developing corneal guttata at a more than two-fold [11]. The aim of the study is to reveal the effect of cigarette smoking on corneal endothelial cells.

2. Patients and Methods

2.1. *Patients*

Study design, a case control cross sectional study. **Subjects,** our study included 99 persons, 61 chronic cigarette smokers and another age matched 38 control non smokers. **Setting,** ophthalmology department - Sohag faculty of medicine. **Inclusion criteria: *** Persons aged 20-50 years. * Cases are chronic smokers for at least 5 years and at least 10 cigarettes a day. **Exclusion criteria:** Any ocular problem such as corneal opacity, DED, glaucoma, uveitis, high errors of refraction, cataract ……etc. **Ethical Considerations:*** Informed written consent was taken from all cases. * Ethical approval was taken from the local scientific ethics committee of Sohag Faculty of Medicine.

2.2. *Methods*

For all persons the following were done: * Slit-lamp examination. * Best corrected visual acuity (BSCVA). * Intraocular pressure (IOP) assessment. * Topcon Non-contact specular microscopy: was performed for the corneal center for all of the participants. If both eyes were qualified to enter the study, only one of them was selected to perform the specular microscopy. These variables were used in our study: cell density (CD), polymegethism, pleomorphism, the average of cell size, and central corneal thickness (CCT). * Cell density was calculated using the following formula: ***Cell Density (cell/mm^2) = 10^6/ Average cell area.*** * The coefficient of variation (CV) was used to show polymegethism: CV= ***standard deviation of the mean cell area/mean cell area (mm^2)***

2.3. *Statistical analysis*

Data were analyzed using IBM-SPSS version 24 (IBM corporation, Chicago. USA, 2016). Data was expressed as mean, standard deviation (SD), number and percentage. Mean and standard deviation were used as descriptive value for quantitative data. Pearson Chi square test was used to compare percentages between two qualitative variables; Student t test was used to compare the means between two groups, and one-way analysis of variance (ANOVA) test was used to compare means of more than two groups; and Pearson Correlation Coefficient was used to estimate the correlation between two quantitative variables. For all these tests, the results were cons-idered significant when the "p value" was <0.05 and highly significant when the "p value" was <0.001

3. Results

The mean age of our study population was 33.64±9.60 years for smokers and 33.45±9.02 for non-smokers. The two groups were age-matched, with non-significant difference between them. On the other hand, they were not sex matched,

as smokers group were 100% males, compared to only 42.1% among the non-smoker group. This is because active chronic smoking is very rare among females in Upper Egypt. Table (1) summarizes the demographic data of the study groups. All of the corneal measures were better among non-smokers compared to smokers; these differences were significant regarding CD, CV and cell hexagonality (HEX), and non-significant only regarding CCT. Table (1) summarizes the case/control comparison. There were non-significant relation between the type of smoking (cigarette, shisha or combined) and corneal measures, and also between smoking severity and corneal measures. Tables (2) and (3) summarize these comparisons

Table (1) Demographic and fundus details of the patients

		Smokers (N=61)	Non smokers (N=38)	Chi square* T test**	P value
Age	Mean±SD	33.64±9.60	33.45±9.02	0.099**	**0.921(NS)**
Sex	Male	61(100%)	16(42.1%)	45.406*	**<0.001(HS)**
	Female	0	22(57.9%)	-	-
Type of smoking	Cigarette	31(50.8%)	-	-	-
	Shisha	21(34.4%)	-	-	-
	Combined	9(14.8%)	-	-	-
No. of smoking per day^	Mean±SD	38.69±36.15	-	-	-
	Median(range)		-	-	-
CCT	Mean±SD	509.97±31.16	517.79±40.64	1.079**	**0.283(NS)**
CD	Mean±SD	2829.9±334.9	3243.6±237.9	6.638**	**<0.001(HS)**
CV	Mean±SD	44.13±9.73	32.84±2.97	6.940**	**<0.001(HS)**
HEX	Mean±SD	40.44±6.95%	60.11±5.83%	14.538**	**<0.001(HS)**

^ *taking in consideration that 1 shisha stone equals 10 cigarettes*

Table (2) Smoker type comparison

	Cigarette Smokers (N=31	Shisha Smokers (N=21)	Combined Smokers (N=9)	ANOVA	P value
Age	32.68±10.21	34.67±9.12	34.56±9.21	0.310	0.735(NS)
CCT	513.00±29.19	510.00±33.25	499.44±34.11	0.652	0.525(NS)
CD	2918.06±290.97	2744.48±409.51	2725.22±207.49	2.291	0.110(NS)
CV	44.61±12.43	43.90±6.34	43.00±5.32	0.101	0.904(NS)
HEX	39.68±7.95%	42.00±6.52%	39.44±3.09%	0.802	0.453(NS)

Table (3) Correlation with smoking severity

	Pearson Correlation	P value
Age	0.236	0.067(NS)
CCT	0.177	0.173(NS)
CD	-0.169	0.192(NS)
CV	-0.018	0.892(NS)
HEX	-0.002	0.990(NS)

4. Discussion

Any condition which causes oxidative stress has harmful effects on different tissue organs, including the corneal endothelium. Smoking introduces a large number of free radicals into the body, hence causing peripheral vasoconstriction and impairs tissue oxygenation [12]. Nicotine, even in low concentrations, stimulates both sympathetic and parasympathetic nervous system; with higher effect on the sympathetic one. This leads to tachycardia and increased peripheral resistance. It also stimulates adrenal gland causing more cardiovascular effects [13]. Carbone

monoxide (CO) is as high as 100folds higher in smokers comparing with non-smokers; leading to increment of carboxyhemoglobin and increases tissue hypoxia [13,14]. The corneal endothelial layer plays an indispensable role in keeping the corneal transparency and preservation of vision [15]. Endothelial cell study is an important step to evaluate corneal function and viability [16-19]. There is an increased need for detailed information on the health of the corneal endothelium. It can be evaluated by the specular microscope and some computer software used to analyze the images; to estimate its thickness and morphology [15]. Endothelial cell parameters including CCT, CV, AVG, CD, and hexagonality could be evaluated by specular microscopy, which could be performed by two methods; contact and non-contact [20]. The cell density (CD) starts to be around 4000 cell/mm^{2}in early childhood, and then declines gradually with age, reaching to only around 2400-2600 cell/mm^2 over the age of 75 years in normal population [13,20]. The normal endothelial cells have hexagonal and regular shapes [21]. Many studies evaluated the corneal endothelial thickness and functions in different health-related conditions. However, there are few comprehensive studies investigating the effects of smoking on the corneal endothelium in the literature [11,22]. Our study revealed that the mean age of our study participants were around 33.5 years, with non-significant differences between smokers and non-smokers. These figures were more or less similar to those of Sayin et al. [23], as the mean age of their study population was 35.5 years among smokers and 34.7 years among non-smokers. This was also agreed by Ilhan et al. [24], where the mean age was 31.5 years among smokers and 33 years among no smokers. On the other hand, our participants were younger than those studied by Golabchi et al. [13] where the mean age of smokers was 48.5 years, and non-smokers was 46.5 years. On the other hand, all of our study cases (smokers) were males, compared to only 42% among non-smokers. This may be due to the fact that smoking is very rare among females in Upper Egypt. In the study from Iran done by Golabchi et al. [13] males accounted for more than 97% of both groups. In the study done by Ilhan et al. [24] and Sayin et al. [23], there was male predominance among smokers, but with non-significant difference with the non-smoker group. The type of smoking in our study was cigarette alone in half of the cases (51%), shisha alone in 34% of cases and combined cigarette and shisha in 15% of cases. Our results found that, among the corneal parameters, CD, CV and hexagonal cell percentages were significantly different among smokers compared to non-smokers. Regarding CD, our study revealed a highly signi-ficant difference between cases (2829.9± 334.9 cells/mm^2) and controls (3243.6± 237.9 cells/mm^2). In the study of Golabchi et al. [13], these figures were 2522.67± 307.16 cells/mm^2 versus 2619.30± 258.55 cells/mm^2, with a significant difference (p=0.011). Also, Ilhan et al. [24] found that the mean CD was significantly lower among smokers (2681±323.9 cell/mm^2) compared to non-smokers (2.881±293.7 cell/mm^2). However, Kara et al. [15] showed non-significant relation between smoking and CD (p=0.441). The CV showed also a highly significant difference between smokers and non-smokers (44.13±9.73 versus 32.84±2.97; respectively, p value <0.001). This was not the case in the study done by Golabchi et al. [13] and Ilhan et al. [24] who failed to establish significant relation between smoking and CV. Similarly, the mean HEX was significantly lower among our cases (40.44±6.95%) compared to non-smokers (60.11±5.83%), p value <0.001. This was similar to the study done by Sayin et al. [23] who reported a significant impairment of HEX among smokers. However, these results were not agreed by Golabchi et al., [13] and Ilhan et al. [24] who found non-significant

difference between cases and controls regarding HEX. On the other hand, our results did not find any significant difference between the two groups regarding average cell size (AVG). Our results were agreed with that of Kara et al. [15] who showed non-significant relation between smoking and CD (p=0.156). This was also agreed by Sayin et al. [23] but not by agreed by the study of Golabchi et al. [13] who found a significant difference between the two groups regarding AVG (402.61±52.53 μm^2 versus 383.62±35.91 μm^2 among cases and controls; respectively with p=0.039). Also, the results of comparing corneal parameters with age, type or severity of smoking in our study revealed non-significant correlations, p values >0.05. The study done by Golabchi et al. [13] found a strong positive and significant correlation between age and AVG (r= 0.539, p <0.001), a strong negative and significant correlation between age and CD (r= -0.547, p <0.001). Chronic smoking increases reactive oxygen metabolites in the blood, causes oxidative damage in the tissues [25] and increase the level of oxygen radicals and also decreases the levels of ascorbic acid, an important antioxidant; in the aqueous humor and different eye tissues [26]. The avascular corneal tissue is also affected by the surrounding ischemia and hypoxia [27], and the effects of smoking-related ocular hypoxia and oxidative stress can put the corneal endothelium at risk [15]. Also, chronic hypoxia may decrease the percentage of hexagonal endothelial cells [28-30]. This hypothesis was supported by some studies which have reported an increased risk of guttata development and morphological changes in the corneal endothelium [11,23]. In the study of Zoega et al. [11], they reported that smoking of more than 20 packs per year increases the risk of developing corneal guttata over two-folds. On the other hand, Sopapornamorn et al. [22] did not find any smoking-related endothelial cell damage. Also, Sayin et al. [23] reported that the mean endothelial cell density and the coefficient of variation in ell size were not significantly different between smoker and non-smoker groups; but they found that smokers had a significantly lower hexagonal cells percentage than non-smokers. This could be explained by the chronic hypoxia in the smokers group. The study of Kara et al. [15] also found that there were no significant differences between the smoker and non-smoker groups for the mean endothelial density, percentage of hexagonal cells, percentage of cells with an area larger than 400 mm^2, or the coefficient of variation of size. Also, they found non-significant differences among smokers regarding corneal endothelial parameters according to the severity of smoking. These opposing results may be due to differences between the groups in these studies and not separating passive smokers [31]. For example, the study of Sopapornamorn et al. [22] which failed to show any significant relation had already studied subjects with lower smoking index (less than 20 packs/year); a factor which may have minimized any difference that may have existed [15,23].

5. Conclusion

Cigarette smoking has deteriorating effects on some, but not all, of the corneal endothelial measures, with no relation found between this deteriorating effect and both of the dose or type of smoking.

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